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Front Cover: Grasshopper caught in *Drosophylum lusitanicum*. Photo by Chiara di Biase. Article on page 88.

Back Cover: Massive stands of *Sarracenia leucophylla* at Splinter Hill Reserve. Note variation amongst pitchers. Photo by Brian Barnes. Article on page 68.

Carnivorous Plant Newsletter is dedicated to spreading knowledge and news related to carnivorous plants. Reader contributions are essential for this mission to be successful. Do not hesitate to contact the editors with information about your plants, conservation projects, field trips, or noteworthy events. Contributors should review the "Instructions to Authors" printed in the March issue of each year. Advertisers should contact the editors. Views expressed in this publication are those of the authors, not the editorial staff.

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Contents

Due to a printer error, several lines on page 51 in the article "The Folklore of Man-Eating Plants" were overprinted and unreadable. We apologize for the error. The complete paragraph is below:

> Travelers have told us of a plant, which they assert grows in Central Africa and also in South America, that is not contented with the myriad of larger insects which it catches and consumes, but its voracity extends to making even humans its prey. This marvelous vegetable Minotaur is represented as having a short, thick trunk, from the top of which radiate giant spines, narrow and flexible, but of extraordinary tenaciousness, the edges of which are armed with barbs, or dagger-like teeth. Instead of growing upright, or at an inclined angle from the trunk, these spines lay their outer ends upon the ground, and so gracefully are they distributed that the trunk resembles an easy couch with green drapery around it. The unfortunate traveler, ignorant of the monstrous creation which lies in the way, and curious to examine the strange plant, or to rest himself upon its inviting stalk approaches with out a suspicion of his certain doom. The moment his feet are set within the circle of the horrid spines, they rise up, like gigantic serpents, and entwine themselves about him until he is drawn upon the stump, when they speedily drive their daggers into his body and thus complete the massacre. The body is crushed until every drop of blood is squeezed out of it and becomes absorbed by the gore-loving plant, when the dry carcass is thrown out and the horrid trap set again.

Splinter Hill Reserve,
an in-depth look

Brian Barnes • ICPS Conservation Director • brian@carnivorousplants.org

Keywords: Splinter Hill Reserve, Conservation

I arrived in beautiful Baldwin County, Alabama on the misty morning of May 8th, 2009. Heavy blankets of fog cascaded their way across the vast bog expanses and the humidity was literally thick enough to cut with a knife. And that was when I saw them...Rolling seas of snowy-white, barely discernable through the drifting clouds, as if they were peeking through from some sort of magical dreamland. This was it. This was what I had just driven nine hours for. I'd seen photos through the years of massive ponds of *Sarracenia leucophylla*, but only was familiar with the ever-dwindling smaller colonies of the aforementioned that existed in the Florida Panhandle. But never anything like this. This was serious "Leuco" country and I knew it. (see Back Cover)

The hair stood on my neck as I envisioned what the next three days in paradise was gonna be like!

I looked forward to meeting with Keith Tassin of The Nature Conservancy (TNC) and interviewing him on video. As Director of Conservation for the ICPS, I was here on a mission. The ICPS has been a frequent supporter of TNC's wonderful efforts at maintaining and guarding this pristine environment and I was here to see what bang we were getting for our buck. From what I saw, we definitely had never wasted one dime of it! I was *very* impressed by what I saw.

As I pulled up to our rendezvous point, I couldn't stand it any longer! Burdened with loads of camera gear, I just had to get a few shots in as the fog was beginning to lift and the lighting was perfect. Finishing my last sips of coffee, I stared in sheer amazement at the incredible diversity amongst the massive *Sarracenia*

Figure 1: Beautiful Splinter Hill Reserve in Alabama, USA. Rain collection gauges can be seen in the left background as part of University of Southern Alabama's genetics research project. All photos by Brian Barnes.

colonies. Beautiful stands of *Sarracenia leucophylla*, *Sarracenia rubra* subsp. *wherryi*, *Sarracenia psittacina* and *Sarracenia rosea* all were existing in perfect harmony and there were very complex hybrids between all four mentioned species present. Upon seeing this, I truly must say I question the species purity of most of the *Sarracenia* in my own collection! Many of the populations present, showed some sort of genetic tainting of species purity. I've mainly been studying *Sarracenia minor* populations in central Florida over the last several years as a personal preference, because they are hundreds of miles from any other *Sarracenia* species, and species purity is most definitely maintained in those colonies.

Surprisingly enough, there were a few hybrids with blatant *Sarracenia flava* characteristics, although a full day's search revealed no *S. flava* stands present. Quite clearly, *S. flava* were here at one time and their obvious influence can be plainly seen in the Figures. I was eager to begin the day's filming. Keith Tassin of TNC gave me the "Tour de Grande" on the first day and I was very impressed with his knowledge of the area. Through the much needed prescribed fires that the ICPS helps to fund with the generous donations from its cherished members, Splinter Hill Reserve has remained unspoiled and is truly one of the finest *Sarracenia* locations in the U.S. And now, it was time to get to work!

I began walking amongst the tall colonies of *Sarracenia* that were so thick that my pant legs smelled like nectar afterwards. My goal was to try my very best to film an up close and personal encounter with this enchanted place in order to raise public awareness, which also leads to conservation. But I am also well aware that there must be a fine balance between the two. I tried to capture every possible natural sound of the birds and insects that inhabit this magical land. Sometimes this required lying in the mud in order to get up close and personal, but I didn't mind. It was all for the future well-being of our beloved CP! I tried my best to bring the actual full bog experience to people around the world, who may never get a chance to actually see the magnificent wonder of this place.

The ICPS has also funded genetic research going on at Splinter Hill by the University of Southern Alabama. While I was there, I saw the plots, markers and water collectors that were set up for gathering data. Unfortunately, very foul weather in the region shortly after my visit delayed the end results of this fine work for a bit. But I am in constant touch with the U. of S.A. and will be reporting the results to the ICPS and our members first-hand, as soon as they are complete.

Figure 2: A fabulous *Sarracenia flava* x *leucophylla* hybrid. Photo by Brian Barnes.

For the last two days, I flew solo. Upon our departure, I graciously thanked Keith Tassin and TNC for their expertise and unbeatable hospitality during my filming and research. The remaining days flew by way too fast. I was in the field 12 hours a day and loving every minute. It was almost like being in another world. A world full of hungry open mouths swaying gently in the breeze, waiting to be fed.

On the day of my leaving, I was still in a daze of amazement from the diversity amongst the *Sarracenia* colonies and the pristine conditions of the bogs. I wish I had more time, but there were more fish to fry, so to speak. I was meeting with Mark Todd of the North American *Sarracenia* Conservancy (NASC) and Serge Grondin in the magnificent Florida Panhandle to aid in a CP rescue from a condemned site in Bay County. Many plants were rescued during this trip and full video documentation of the ICPS/NASC CP Rescue and the Splinter Hill Experience can be viewed in HD via the link on the ICPS homepage under "ICPS Projects"

After the CP rescue operation was finished, it was time to show some of my old familiar CP haunts to Mark and Serge in the Florida Panhandle. So once again with camera bags in hand, we were off!

Stay tuned for details in the next wonderful CPN issue! Until then, Happy Growing!

Brian Barnes, ICPS Director of Conservation.

Figure 3: A rare red form of *Sarracenia leucophylla*. Photo by Brian Barnes.

Ecophysiological investigation on *Drosophyllum lusitanicum*: Why doesn't the plant dry out?

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Keywords: physiology: *Drosophyllum lusitanicum*, roots.

Introduction

Drosophyllum lusitanicum (L.) Link (Portuguese dewy pine; Droseraceae) is the only carnivorous plant with distinctly xerophytic features even during the growing season, in great contrast to the general strategy in other carnivorous plants (Givnish *et al.* 1984; Juniper *et al.* 1989). It grows sporadically in a limited area in the subtropical Southern and Western parts of the Iberian Peninsula in Spain and Portugal as well as at the northernmost tip of Africa in Morocco (*e.g.*, Müller & Deil 2001; Garrido *et al.* 2003). *Drosophyllum* is a perennial herb (or short-lived subshrub; Carlquist & Wilson 1995) with a woody stem base which may be up to 1 cm (0.4 in) thick and poorly branched. Adult plants may be up to 90 cm (35.5 in) high. The narrow linear leaves are 15-20 cm (5.9-7.9 in) long and bear numerous immobile emergences (tentacles) with glands and droplets of sticky mucilage (Juniper *et al.* 1989). The exact morphology of the root is almost unknown; only a relatively thick woody taproot has been described. Guttenberg (1968; see also Adlassnig *et al.* 2005) presented cross-sections of lateral roots of *Drosophyllum* and pointed out some peculiarities: the secondary endodermis is heavily suberized, while the rhizodermis is lignified. Carlquist & Wilson (1995) classified the wood anatomy of *Drosophyllum* roots as xeromorphic.

Drosophyllum occurs in an area with very hot and seasonally arid conditions, with summer periods of up to three months without rain. Temperatures of the air or the topsoil within plant stands may exceed 40ºC (104ºF) (Adlassnig *et al.* 2006). However, during the night, very high air humidity can result in dew. As a facultative heliophyte, *Drosophyllum* grows preferentially in open habitats without close vegetation, in heathlands and open stands of trees or shrubs (Müller & Deil 2001). At these sites, the soil is derived from sand or sandstone and is therefore typically rich in large particles (rock or coarse sand), but acidic and poor in organic matter and mineral nutrients (Müller & Deil 2001; Correia & Freitas 2002; Garrido *et al.* 2003; Adlassnig *et al.* 2006).

In spite of unfavorable climatic and soil conditions, the plants in the field are green, turgescent, and produce trapping mucilage on their leaves even in the middle of the dry season (Adlassnig *et al.* 2006). However, it is still unclear which principal ecophysiological strategy is used by *Drosophyllum* to ensure a sufficient water supply and, secondly, which adaptations are used to withstand successfully the very high temperatures in summer. Although a great deal of speculation has been made on ecological peculiarities of *Drosophyllum*, few specific publications on this subject have been published (Carlquist & Wilson 1995; Müller & Deil 2001; Correia & Freitas 2002; Garrido *et al.* 2003; Adlassnig *et al.* 2005, 2006).

Theoretically, there are two possibilities to explain how *Drosophyllum* maintains its turgescent characters in such conditions: (1) The plant could use a very efficient system of water uptake via deep reaching roots (the natural root system is said to be over 1 m (40 in) deep!; Hampe, pers. comm.) to cover a relatively great transpiration water loss or (2) the root system is rather inefficient and, thus, shoot transpiration is greatly reduced like in succulents (*sensu* Lambers *et al.* 1998). A preliminary observation on *Drosophyllum* root morphology is ambiguous (Adlassnig *et al.* 2006). It is evident that the plant absorbs water condensed onto the leaves (probably mainly on hygroscopic drops of mucilage on the tentacle heads) from night fog as dew (*e.g.*, Juniper *et al.* 1989; Adlassnig *et al.* 2006). Yet, in the absence of water-storage tissues or organs, such a limited water supply alone cannot provide the plant with a sufficient amount of water for a "normal" transpiration rate during the whole day. Thus, it is possible to hypothesize that transpiration of leaves is greatly reduced by a thick cuticle and low density of stomata. Such adaptations are common among Mediterranean shrubs with evergreen scleromorphic leaves, which grow in similar hot and dry habitats. This feature is associated with a relatively low photosynthetic rate, low stomatal conductivity, and a high osmotic value in leaf cells (Lambers *et al.* 1998). In this line, a relatively low maximum net photosynthetic rate of only about 6 μmol CO_2 m^{-2} s^{-1} has recently been found in leaves of outdoor-grown *Drosophyllum* plants (Hájek & Adamec, unpublished data), while common values for leaves of herbs lie between about 20-45 μmol CO_2 m^{-2} s^{-1} (*cf.*

Lambers *et al.* 1998). However, a low osmotic value of only about 500 mOsmol was found in the field-grown plants (Adlassnig *et al.* 2006).

The aim of this study was to specify soil pH at ten sites of *Drosophyllum* in Spain and Portugal, to document root morphology and root:shoot ratio, and to measure the leaf mineral nutrient content in field-grown plants. Thus, the aim is to specify the mechanisms that enable *Drosophyllum* to withstand hot and arid conditions.

Materials and Methods

All field investigations at *Drosophyllum* sites in Southern Spain or Western Portugal were conducted during 26-30 April 2005. At ten *Drosophyllum* sites, about 15-20 ml of the upper soil layer (0-1 cm) was sampled from 3-5 representative microsites as a mixed sample within each site and stored in a plastic vial for pH measurement. The altitude at these sites ranged from about 35 to 680 m a.s.l. While *Drosophyllum* is protected by conservation laws in Spain, in Portugal the plant is not protected even though it is less abundant and more endangered. Accordingly, our sampling of plants was done only at two sites in Portugal; the exact locations are not presented here for reason of conservation of the stands. Adult, non-flowering plants were carefully dug out from the soil using a small blade; two plants were dug out from a sandy soil in a man-made *Eucalyptus* plantation south of Porto and four plants from a sandy-stony soil in a natural shrub-dominated heathland north of Porto. Great attention was placed upon digging out the entire root systems. In spite of these efforts, it is possible that some final parts of fine lateral roots were lost. The total length of the main root was measured as well as the soil depth in which the root tip occurred. The plants were separated into shoots and roots. The root systems were photographed. The roots were washed with tap water, blotted dry, and air-dried in opened plastic bags. The shoots were treated by first removing all dead leaves and captured prey. Then the shoots were dried out using the same protocol as on the roots.

At the site north of Porto, one adult leaf was cut from each of six adult, non-flowering plants for determination of mineral nutrient content. Using a pair of forceps, all captured prey was removed from the leaves, without touching the leaves with fingers. The leaves were put in a clean plastic bag in which they were air-dried.

All samples were fully dried at 80°C (176°F) and the dry weight (DW) was measured in the laboratory. The proportion of root to total DW was estimated. Dry leaves were ground by a pair of forceps and aliquots of 1-3 mg were weighed for mineralization with mineral acids and subsequent N, P, K, Na, Ca, and Mg measurement by colorimetry or atomic absorption spectrometry (for all analytical details see Adamec 2000, 2002). Four to five parallel leaf samples were analyzed. Leaf nutrient content is expressed in % of DW. Water pH of the collected soils was measured by a pH electrode in soil suspensions (soil:water ca. 1:2 vol./vol., 5 h).

Results and Discussion

At the ten *Drosophyllum* sites in our study, the soil pH ranged from 3.67 to 5.30 (mean pH 4.46; median pH 4.41; SD 0.41). This pH range corresponds to the data reported by other authors (Correia & Freitas 2002; Garrido *et al.* 2003; Adlassnig *et al.* 2006) but is rather within the lower range of these literature data. One can conclude from all available data that *Drosophyllum* prefers acidic soils of a pH between 4-5, but can also grow in strongly acidic as well as in more or less neutral soils (6.2±0.5; mean±SD; Garrido *et al.* 2003). Thus, the soil pH range for *Drosophyllum* growth is rather wide, ca. 3.6-7.0, and confirms some tolerance of neutral soils. In slightly acidic or neutral soils, the bedrock consists of sandy limestone but the soil contains only little water-extractable calcium (Adlassnig *et al.* 2006). Generally, based upon the available mineral nutrient (N, P, K, Ca, Mg) content, the soils can be characterized as mineral-poor (Correia & Freitas 2002; Garrido *et al.* 2003; Adlassnig *et al.* 2006).

The root system of *Drosophyllum* was found to be rather well developed, strongly branched, and very fragile (see Figure 1). Presumably, the fragility of the root system is one of the reasons why this species cannot be transplanted when in cultivation unless still in the seedling stage. Yet, the proportion of fine lateral roots, which participate in taking up mineral nutrients and water, might be estimated at only 3-5% of the total root biomass. The root system has a distinct, heavily lignified taproot which comprises the majority of the biomass. Unexpectedly, the main root was only about 15-37 cm (5.9-14.6 in) long (see Table 1) and reached a depth of only 15-33 cm (5.9-13 in). The root DW was about 23% of the total plant DW. These data correspond to those by Adlassnig *et al.* (2006) who preliminarily found a relatively short root system for *Drosophyllum*. Although the relatively high proportion of root DW in *Drosophyllum* falls into the upper range found in carnivorous plants (compared to *e.g.*, *Drosera adelae*; see Adamec 1997), most other species are wetland plants without woody roots.

Before the roots were dug out, the stem shoots were cut off at their bases and monitored for at least one hour. No water exudation as a result of root pressure was apparent on the root stumps. Taking into account the xeromorphic anatomical structure of roots (Carlquist & Wilson 1995; Adlassnig *et al.* 2005 *ex* Guttenberg 1968), the root system of *Drosophyllum* is evidently not able to supply the plant with sufficient water to cover transpiration rates when the weather is hot and the soil is dry. Thus, other adaptation mechanisms should occur on the shoot level.

The mineral nutrient content in *Drosophyllum* leaves (see Table 2) is comparable to values commonly estimated in leaves of wetland species of terrestrial carnivorous plants (*cf.* Adamec 1997, 2002; Ellison 2006). On the one hand, *Drosophyllum* plants growing in nutrient-poor soils were not limited in their growth by shortage of any mineral macronutrient estimated (N, P, K, Ca, Mg). Evidently, the combination of carnivory (mainly N, P) and the root nutrient uptake (mainly K, Ca, Mg) is sufficient to supply the plants with sufficient mineral nutrients for plant growth (Adamec 1997). On the other hand, leaf K and especially Na content was relatively low and supports the recent finding of relatively low osmotic value in vacuoles of *Drosophyllum* leaf cells (Adlassnig *et al.* 2006). These values demonstrate that this species does not behave as a halophyte, as was suggested by Juniper *et al.* (1989).

Drying of collected shoots and leaves of *Drosophyllum* in small open plastic bags revealed an important feature of its leaves. The cut-off leaves dried out very slowly for many days. Under the same conditions, sticky leaves of other wetland carnivorous plants (*e.g.*, *Drosera, Pinguicula*) dry out very quickly, within 1-2 days. Therefore, we can conclude that *Drosophyllum* leaves are provided with a thick cuticle efficiently preventing water losses by transpiration. Moreover, we expect that the density of stomata on the leaves is low and that the stomata are sunken deeply into the leaf mesophyll. Thus, the adaptation of *Drosophyllum* to very hot and arid climate could be the same as in other co-occurring xerothermic and xeromorphic evergreen Mediterranean shrubs.

Unlike more conventional shrubs with dry leaf surfaces, *Drosophyllum* leaves are provided with numerous tentacles producing sticky mucilage that does not dry out in the hot and dry summer. As reported by Darwin (1875, p. 335-336) when a cultured plant was placed in a jar at 100% air humidity, it produced such a copious amount of constant mucilage secretion that it wetted the plant surface. Thus, it is possible to assume that this mucilage is greatly hygroscopic (unlike most other wetland carnivorous species). During the night, the plants are able to absorb water condensed from oceanic fog as dew onto their mucilage droplets on tentacles. In this way, the mucilage droplets increase their size and water availability (in terms of increased water potential) for the plant overnight and the tentacle heads are able to absorb a part of this condensed water into the leaves. This process of water absorption from the mucilage can stop in the morning, due to

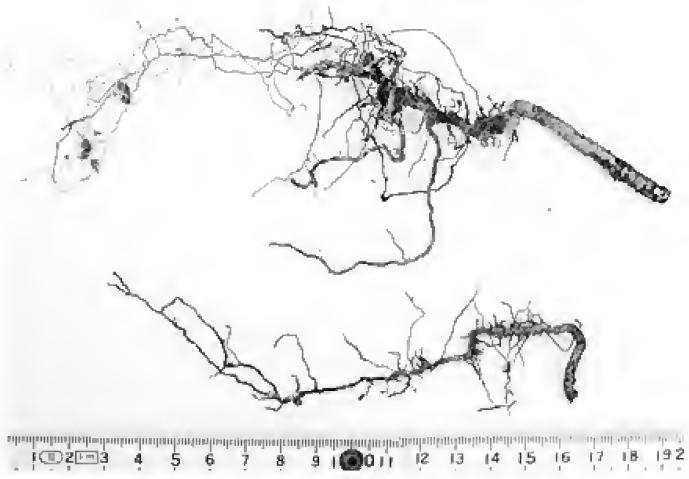

Figure 1: Intact root system of *Drosophyllum*.

Table 1: Parameters obtained on six plants of *D. lusitanicum* collected at two natural sites near Porto, Portugal, on 29-30 April 2005.

	Mean	1 SE	Range of values
Shoot DW (g)	1.02	0.27	0.375-1.97
Root DW (g)	0.265	0.052	0.173-0.496
Root: total biomass (%)	23.3	3.4	14.2-38.0
Main root length (cm)	22.2	3.9	15-37
Approx. soil depth at the root tip (cm)	17.6	4.0	10-33

Table 2: Mineral nutrient content (in % of DW) in adult leaves of *D. lusitanicum* collected at natural sites S (2 plants) and N (3 plants) of Porto, Portugal, on 29-30 April 2005. Means±1 SE are shown; n=4-5.

N	P	K	Na	Ca	Mg
1.75±0.14	0.179±0.032	1.10±0.09	0.068±0.022	0.300±0.040	0.151±0.017

increasing air temperature and decreasing relative humidity. Only in the morning, stomata are open, and the rate of photosynthesis can be relatively high. Later, under very hot and dry conditions at noon and afternoon, stomata are already closed and both photosynthetic and transpiration rates are very low ("noon depression of photosynthesis"). Under these conditions, the transpiration stream from the roots is probably zero. As Adlassnig *et al.* (2006) found a significantly lower temperature on the leaf surface than in the ambient air (by 5.5±3.8°C (42.9±6.8°F)) during daytime, some minimum transpiration rate is maintained even during hot and dry afternoons. The source of the water for this transpiration can be (1) the mucilage, (2) the water stored in the leaves, and (3) the relatively thick wooden stems.

To confirm this hypothesis, direct field measurement of transpiration rate would be useful. Or at least, a desiccation curve estimated in a laboratory on a cut-off plant shoot could distinguish between transpiration of the mucilage and the leaf. Finally, a determination of cuticle thickness as well as density and anatomy of stomata would demonstrate whether the above hypothesis on the essence of adaptation to hot and dry climate is valid.

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Comparative studies on the acid proteinase activities in the digestive fluids of *Nepenthes, Cephalotus, Dionaea,* and *Drosera*

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Keywords: chemistry: *Nepenthes, Cephalotus, Dionaea, Drosera*

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Introduction

There are various kinds of carnivorous plants in nature, and most of them excrete acidic digestive fluids which contain digestive enzymes, especially acidic proteinases, to digest trapped insects and other prey for nutrition (Juniper *et al.* 1989). Previous inhibitor studies (Lobareva *et al.* 1973; Takahashi *et al.* 1974; Tökés *et al.* 1974) indicated that the acid proteinase nepenthesin from *Nepenthes* belongs to the aspartic proteinase family. Recently, we purified two nepenthesins from the digestive fluid of *N. distillatoria*, investigated their molecular and enzymatic properties, and elucidated their primary structures by cDNA cloning of nepenthesins from *N. gracilis* (Athauda *et al.* 1998, 2002, 2004; Takahashi *et al.* 2005). The peptide bond cleavage specificity of nepenthesin was investigated using *N. alata* pitcher fluid (Ann *et al.* 2002), partially purified nepenthesin from a mixture of several *Nepenthes* species (Amagase *et al.* 1969) and from *N. macfarlanei* (Tökés *et al.* 1974), and fully purified nepenthesin from *N. distillatoria* (Athauda *et al.* 2004; Takahashi *et al.* 2005). In addition, some enzymatic properties of the acid proteinase activities in the crude digestive fluids of *Nepenthes* sp. (Lüttge, 1964) and *Dionaea muscipula* (Scala *et al.* 1969; Robins & Juniper 1980) and a partially purified acid proteinase from *Drosera peltata* (Amagase *et al.* 1972a, 1972b) were reported. Except for these studies, however, not many studies have been performed on the enzymatic properties of these acid proteinases. Therefore, further studies are necessary to understand more extensively the nature of the acid proteinases in the digestive fluids of carnivorous plants. Since the digestion takes place in the crude digestive fluid, it is thought to be important to characterize the acid proteinase activity as a whole as well as to purify and characterize the individual proteinases. Thus, we have investigated in the present study some enzymatic properties of the acid proteinase activities in the crude digestive fluids of typical carnivorous plants including two pitcher plants, *Nepenthes alata* and *Cephalotus follicularis*, one plant with a snap-trap, *Dionaea muscipula* (Venus flytrap), and one plant with a mucilage trap, *Drosera capensis* (sundew), in a comparative manner. The results demonstrated significant differences among them, presumably reflecting the phylogenic diversity of these carnivorous plants.

Materials and Methods

The crude digestive fluids of *Nepenthes alata* and *Cephalotus follicularis* were collected in the Daishoen plantation (Numazu). Dionea *muscipula* and *Drosera capensis* were obtained from the Daishoen plantation and grown at the botanical garden of Tokyo University of Pharmacy and Life Sciences to obtain their digestive fluids. The digestive fluid of *Dionaea* was collected 3 to 4 days after giving a piece (about 3-5 x 3-5 x 2-3 mm) of boiled egg white, thereby inducing closure of the two lobes by stimulating the trigger hairs. The digestive fluid of *Drosera* was collected by soaking ten leaves successively (1 leaf for 1 min at a time) in 800 µl of 0.1 M sodium acetate buffer, pH 4.0, in a test tube to wash out the digestive fluid through up-and-down strokes. The pH was determined using a glass electrode in a Horiba pH-meter. The samples were kept frozen at -20°C

until use. Porcine pepsin A, bovine hemoglobin, the B chain of oxidized bovine insulin and leucyl-4-methylcoumaryl-7-amide (Leu-MCA) and arginyl-4-methylcoumaryl-7-amide (Arg-MCA) were obtained from Sigma and pepstatin A from Peptide Institute, Osaka. Other reagents used were of analytical grade.

The proteinase activities of the digestive fluids of *Nepenthes*, *Cephalotus*, and *Dionaea* were determined with bovine hemoglobin as a substrate at pH 2.0 as described (Athauda *et al.* 1989), and expressed in pepsin equivalent using porcine pepsin A (Sigma) as a standard. The proteinase activity of the digestive fluid of *Drosera capensis* in the hemoglobin assay was markedly low as compared with those of the other three. Therefore oxidized insulin B chain was used as a substrate and the digestion products were analyzed by HPLC. The assay mixture was composed of 1-40 µl of enzyme solution, 10 µl of oxidized insulin B chain (1 mg/ml), 50 µl of 0.1 M sodium acetate buffer, pH 4.0, an appropriate volume (0-39 µl) of water in a total volume of 100 µl, and the digestion was performed at 37°C for 1 h and terminated by the addition of 100 µl of 0.5 M H_3BO_3-KCl/NaOH buffer, pH 9.8. A 100-µl aliquot from the reaction mixture was analyzed by reverse-phase HPLC using a Shimadzu LC10A system on a Tosoh ODS-120T column (4.6 x 250 mm) eluted with an acetonitrile gradient (0 to 60%) in 0.1% trifluoroacetic acid at a flow rate of 1.0 ml/min and monitored at 215 nm. The activity was estimated from the decrease in the amount of the substrate, and expressed in pepsin equivalent determined using porcine pepsin A as a standard under the same assay conditions at pH 4.0.

Aminopeptidase activities were determined with Leu-MCA and Arg-MCA as substrates. The assay mixture contained 5 µl (*Nepenthes*), 200 µl (*Cephalotus*), or 10 µl (*Dionaea*) of enzyme solution, 5 µl of 10 mM substrate in dimethylsulfoxide, 310 µl of 0.1 M citric acid-Na_2HPO_4 buffer, pH 3.0-6.0, and an appropriate volume (0-195 µl) of water in a total volume of 515 µl. The digestion was performed at 37°C for 30 min and stopped by the addition of 2.5 ml of 5% trichloroacetic acid. The amount of 7-amino-4-methylcoumarin liberated was measured in a Hitachi spectrofluorometer with excitation at 380 nm and emission at 460 nm.

Native electrophoresis followed by activity staining was performed as follows. An appropriate portion (20 µl) of each pitcher fluid was submitted to native polyacrylamide gel electrophoresis using 10% acrylamide gel and Tris-glycine buffer, pH 8.7, and then proteinase activity was examined by activity staining with hemoglobin as a substrate at pH 1.7 essentially as described (Furihata *et al.* 1972).

To investigate the substrate specificity, oxidized insulin B chain was used as a substrate. The reaction mixture contained 10 µl (*Nepenthes*), 40 µl (*Cephalotus*), or 3 µl (*Dionaea*) of the digestive fluid containing about 1 pmol enzyme as porcine pepsin equivalent, 10 µl of oxidized insulin B chain (1 mg/ml), 50 µl of 0.1 m sodium formate buffer, pH 3.0, and an appropriate volume (0-37µl) of water in a total volume of 100 µl. The digestion was performed at 37°C for 3 h, and the resulting peptides were analyzed using an HPLC apparatus (1100 series, Agilent Technology) with a TSKgel ODS-120T column (2.2 x 150 mm) connected to an LC™-DUO mass spectrometer (ThermoQuest). Amino acid sequences of the peptides produced were determined from the mass spectra of the original and fragmented ions by using Xcalibur Bioworks 1.0 software installed in the apparatus as described (Nishii *et al.* 2002).

Results and Discussion

The collected fluid samples of *Nepenthes alata*, *Cephalotus follicularis*, and *Dionaea muscipula* had a pH of 2.9, 2.9, and 3.9, respectively, and were shown to contain approximately 10 pmol, 2 pmol, and 40 pmol, respectively, of acid proteinase in pepsin equivalent per 100 µl as determined with hemoglobin as a substrate. On the other hand, the digestive fluid of *Drosera capensis* had a pH of about 2.5, and the washate of ten leaves with 800 µl of the pH 4.0 buffer contained approximately 100 pmol of acid proteinase in pepsin equivalent per 100 µl as determined with oxidized insulin B chain as a substrate.

Figure 1 shows the activity staining after native polyacrylamide gel electrophoresis (PAGE) of the crude digestive fluids. Two broad bands are seen for the *Nepenthes* sample; the major, slow-moving band should correspond to nepenthesin I and the minor, fast-moving band to nepenthesin II. These broad bands may contain more then one acid proteinase isozyme as shown for nepenthesins I and II from *N. gracilis* and *N. distillatoria* (Athauda *et al.* 2004; Takahashi *et al.* 2005). The *Dionaea* sample gave a single broad band moving almost in parallel with nepenthesin I, but the band corresponding to nepenthesin II was hardly seen. The major band may contain more than one acid proteinase component. Indeed, it was reported previously that two major bands were detected on native PAGE of the digestive fluid of *Dionaea muscipula* (Robins & Juniper 1980). In the case of *Cephalotus*, there are two bands comparable with the nepenthesin bands, but the major band corresponding to nepenthesin I had higher mobility toward cathode. Thus, the electrophoretic patterns are similar but not identical among the three samples. The analysis of the *Drosera* sample gave no clear band presumably due to the low sensitivity of the enzyme toward hemoglobin (data not shown).

Figure 2 shows the pH profiles of the enzymatic activities. Both the *Nepenthes* and *Cephalotus* samples had the pH optimum at around 2.5, whereas the *Dionaea* and *Drosera* samples showed the pH optimum at 3.0 and 3.5, respectively. The result with the *Nepenthes* sample is similar to the pH profiles of nepenthesins I and II from *N. distillatoria* having an optimum at pH 2.6 and that of the crude fluid from *N. distillatoria* with an optimum at pH 2.8 (Athauda *et al.* 2004; Takahashi *et al.* 2005). As compared with the pH profile of the *Cephalotus* sample, those of the other three had broader pH profiles, which might mean that they are composed of more than one enzyme component with fairly different pH optima. As for the *Dionaea* digestive fluid, the pH-activity profile with casein as a substrate was reported to show two peaks at pH 4.0 and 5.0 (Robins & Juniper 1980) or one sharp peak at pH 5.3 (Scala *et al.* 1969). Several pH optima (Chandler & Anderson 1976) or an irregular pH profile (Clancy & Coffey 1977) with casein as a substrate were reported for the digestive fluids of *Drosera* species. The reason for the differences between the present results and those reported previously is not clear at present, but might be at least partly due to the difference in the assay conditions, especially in the substrate used.

The temperature dependence of activity of each sample is shown in Figure 3a. The *Nepenthes* sample showed a temperature-activity profile with a maximum at about 57°C. This is similar to that of nepenthesin I from *N. distillatoria* which has a maximum at 55°C, but the maximum temperature was higher than that of the crude fluid from *N. distillatoria* with a maximum at 50°C (Athauda *et al.* 2004; Takahashi *et al.* 2005). The results are roughly similar to that reported for the digestive fluid of *N. khasiana* (Lüttge 1964). On the other hand, the *Cephalotus*, *Dionaea*, and *Drosera* samples showed a temperature-activity profile with a maximum at 47°C, 47°C, and 42°C, respectively, which are apparently similar to that of nepenthesin II from *N. distillatoria* (Athauda *et al.* 2004; Takahashi *et al.* 2005). The profile of the *Dionaea* sample has a shoulder at around 60°C, which might indicate the presence of more than one component with fairly different temperature dependence of activity.

The results of temperature-stability experiments are shown in Figure 3b, in which each crude sample was incubated at different temperatures for 1 h, then the remaining activity was determined. As for the *Nepenthes* sample, the activity was stable up to around 53°C, then started to decrease and was lost completely at around 80°C. This result was similar to those obtained with nepenthesin I and the crude fluid from *N. distillatoria* (Athauda *et al.* 2004; Takahashi *et al.* 2005). A similar result was obtained with the

Figure 1:

Native polyacrylamide gel electrophoresis with proteinase activity staining of the digestive fluids of carnivorous plants. (a) *Nepenthes alata,* (b) *Dionaea muscipula,* (c) *Cephalotus follicularis.*

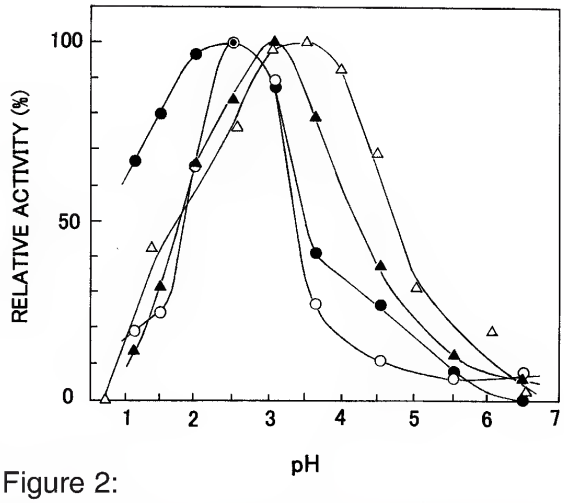

Figure 2:

pH dependence of the proteinase activities of the digestive fluids of carnivorous plants. Closed circle, *Nepenthes alata*; open circle, *Cephalotus follicularis*; closed triangle, *Dionaea muscipula*; open triangle, *Drosera capensis*. The same symbols are used in Figures 3-5. Buffers used were 0.1 M HCl/KCl buffers (pH 1.1 to 2.0) and 0.1 M citric acid/Na_2HPO_4 buffers (pH 2.5 to 6.5) except for *Drosera* for which 0.1 M HCl/KCl buffers (pH 0.7 to 1.4), 0.1 M glycine/HCl buffers (pH 2.6 to 3.5), 0.1 M potassium acetate/HCl buffers (pH 4.0 to 5.4) and 0.1 M KH_2PO_4/NaOH buffers (pH 6.1 to 6.6) were used.

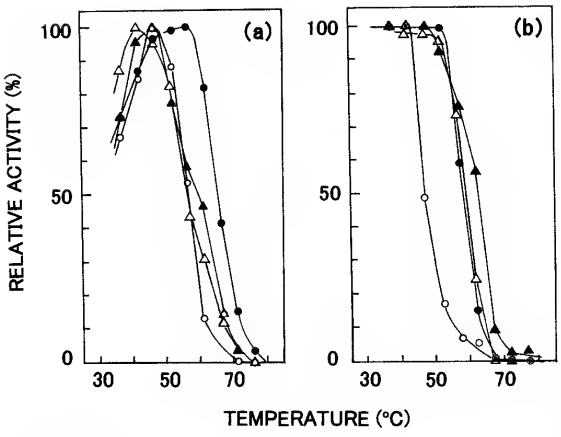

Figure 3:

Effects of temperature on the proteinase activities and stabilities of the digestive fluids of carnivorous plants. (a) The activity was measured at various temperatures at pH 2.0 except for the *Drosera* sample which was assayed at pH 4.0. (b) Each sample was incubated at various temperatures and pH 2.9 (*Nepenthes* and *Cephalotus*), 3.9 (*Dionaea*), or 4.0 (*Drosera*) for 1 h at an enzyme concentration of approximately 5-10 pmol pepsin equivalents per 200 μl and then assayed as above.

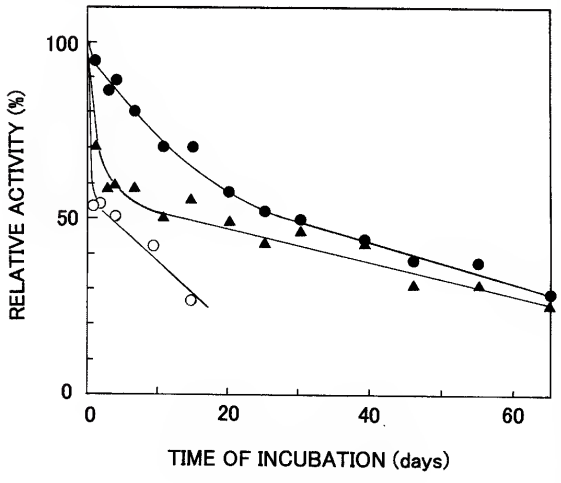

Figure 4:

Stabilities of the proteinase activities of the digestive fluids of carnivorous plants on long incubation. The activity was measured at 37°C after incubation of each sample for various periods at 37°C and pH 2.9 (*Nepenthes* and *Cephalotus*) or 3.9 (*Dionaea*) at an enzyme concentration of approximately 5-10 pmol pepsin equivalents/200 μl. The experiment with *Drosera* was not performed.

Drosera sample. On the other hand, the activity of the *Cephalotus* sample was less stable. It was stable up to 42°C, where it started to decrease to zero at around 70°C. Interestingly, the *Dionaea* sample was apparently more stable than the *Nepenthes* sample at above 55°C. Thus, the Tm values were approximately 63°C, 59°C, 58°C, and 47°C, respectively, for the *Dionaea*, *Drosera*, *Nepenthes*, and *Cephalotus* samples. The temperature-stability profile of the *Dionaea* sample was different from those of the other three. The activity was stable at around 50°C, but above this temperature the inactivation appeared to proceed in two steps, suggesting the presence of at least two enzyme components different in heat stability. These results are consistent with the temperature dependences of the activities shown in Figure 3a.

Figure 4 shows the changes of the acid proteinase activities upon longer incubation at 37°C. The activity of the *Nepenthes* sample decreased only very slowly on longer incubation; about 60% and 30% of the original activity were still retained after 30 days and 65 days of incubation, respectively. Thus, the activity of the crude digestive fluid of *N. alata* is considerably stable under the conditions used. However, under similar conditions, that of *N. distillatoria* retained nearly full activity after 30 days of incubation (Athauda *et al.* 2004; Takahashi *et al.* 2005). This difference might be due to the difference in the *Nepenthes* species used and/or the difference in the experimental conditions. In contrast to the *Nepenthes* sample, the activity of the *Cephalotus* sample was rather unstable; nearly 50% and 70% of the original activity were lost in 1 h and 15 h, respectively. On the other hand, the *Dionaea* sample appeared to be considerably more stable than the *Cephalotus* sample, but somewhat less stable than the *Nepenthes* sample in the early phase of incubation; about 40% and 25% of the original activity were lost in 3 h and 65 h, respectively. As can be seen from Figure 4, the profiles of the activity change of both *Cephalotus* and *Dionaea* samples appeared to be biphasic. This suggests that each of these samples contains a relatively unstable component and a more stable component. The latter component in *Dionaea* appears to be comparable in stability with the *Nepenthes* sample. The experiment with the *Drosera* sample has not yet been performed.

The effects of pepstatin A on the activity of each sample are shown in Figure 5. Under the conditions used, the activities of all samples appeared to be nearly half lost in the initial phase at a low pepstatin concentration (up to about 1 μM). At higher concentration of pepstatin, the *Nepenthes* and *Cephalotus* samples were inhibited further, but more weakly, and nearly complete inhibition occurred with 120 μM of the inhibitor. On the other hand, the activity of the

Dionaea sample was not inhibited completely; the activity was half inhibited at a low concentration of pepstatin A, but the remaining about 50% of the activity was almost insensitive to pepstatin A and remained uninhibited in the presence of 100 μM inhibitor. In contrast, the *Drosera* sample was inhibited completely at a pepstatin concentration of 5 μM.

These results indicate the following. First, the *Nepenthes* and *Cephalotus* samples each may contain two kinds of enzymes with a higher and a lower affinity to pepstatin. Indeed, nepenthesins I and II of *N. distillatoria* have been shown to have different affinity to pepstatin (Athauda *et al.* 2004; Takahashi *et al.* 2005). Second, the *Dionaea* sample may contain a pepstatin-insensitive acid proteinase in addition to the nepenthesin-like pepstatin-sensitive acid proteinase. This pepstatin-insensitive enzyme has not yet been identified, but might be similar to glutamic peptidases such as aspergilloglutamic peptidase (Huang *et al.* 2000; Yabuki *et al.* 2004; Sasaki *et al.* 2004) and scytallidoglutamic peptidase (Fujinaga *et al.* 2004; Kataoka *et al.* 2005) or serine-carboxyl peptidases such as physarolisin (Nishii *et al.* 2003a, 2003b) and pseudomonalisin (Wlodawer *et al.* 2001), each of which is known to be insensitive to pepstatin but has an acidic pH optimum. To our knowledge, such an enzyme as a glutamic peptidase or serine-carboxyl peptidase has not been found so far in plants. The occurrence of at least two different types of acid proteinases is consistent with the biphasic character of the *Dionaea* proteinase activity observed already. The pepstatin-insensitive activity in the *Dionaea* sample appeared to be less stable than the pepstatin-sensitive activity when the enzyme sample was stored at 4°C. In contrast to the present results, the acid proteinase activity in the *Dionaea* digestive fluid was previously reported to be insensitive to pepstatin (Robins & Juniper 1980). The reason for this difference is not certain at present, but might partly be due to the occurrence of two different types of acid proteinases. Third, the *Drosera* sample may contain only acid proteinases with a relatively higher pepstatin affinity.

The peptide bond cleavage specificities of the acid proteinases in the *Nepenthes*, *Cephalotus*, and *Dionaea* samples as examined with oxidized insulin B chain as a substrate are shown in Figures 6 through 8. In the *Nepenthes* sample, the major cleavages occurred at Leu^{15}-Tyr^{16} and Phe^{24}-Phe^{25}, and moderate cleavages at Glu^{13}-Ala^{14}, Ala^{14}-Leu^{15}, Tyr^{16}-Leu^{17}, and Tyr^{26}-Thr^{27}. These results are roughly similar to those reported previously for the crude digestive fluid of *N. alata* (Ann *et al.* 2002) and the purified nepenthesin I from *N. distillatoria* (Athauda *et al.* 2004; Takahashi *et al.* 2005). In the latter case, Leu^{6}-$Cys(ox)^{7}$ bond was cleaved to a significant extent, which might be due to species or isozymic difference. In the *Cephalotus* sample, the major cleavages occurred at Leu^{15}-Tyr^{16}, Phe^{24}-Phe^{25}, and Lys^{29}-Ala^{30}, and moderate cleavages at Glu^{13}-Ala^{14} and Ala^{14}-Leu^{15}. In the case of the *Dionaea* sample, the major cleavages occurred at Glu13-Ala14, Leu15-Tyr16, and Phe^{24}-Phe^{25}, and moderate cleavages at Ala^{14}-Leu^{15}, Tyr^{16}-Leu^{17}, Gly^{23}-Phe^{24}, and Lys^{29}-Ala^{30}. Thus, the major cleavages at Leu^{15}-Tyr^{16} and Phe^{24}-Phe^{25} were common, but the other cleavage sites varied significantly among the three samples. Especially, the *Cephalotus* and *Dionaea* samples differ from the *Nepenthes* sample in that Lys^{29}-Ala^{30} and Glu^{13}-Ala^{14}, respectively, are also the major cleavage sites. These differences should reflect the difference in the cleavage specificity of the enzyme components in each sample.

Aminopeptidase activities in the acidic pH range were determined using Leu-MCA and Arg-MCA as substrates. Neither activity was detected with *Nepenthes* and *Cephalotus* in the pH range of 3-6 and 3-5, respectively. As for *Dionaea*, a trace activity toward Leu-MCA was detected but no activity toward Arg-MCA in the pH range of 3-6. Therefore, practically no aminopeptidase action is assumed to be involved in the cleavages of oxidized insulin B chain observed in the present study. On the other hand, the activities of carboxypeptidases were not analyzed in the present study; the possibility for the action of a carboxypeptidase(s) cannot be completely excluded, however, considering the reports of the occurrence of carboxypeptidase in the digestive fluids of *N. alata* (Ann *et al.* 2002) and *Dionaea* (Robins & Juniper 1980).

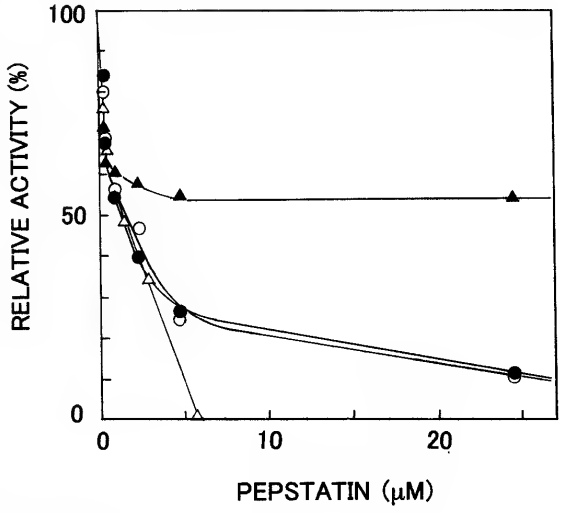

Figure 5:

Effects of pepstatin A on the proteinase activities of the digestive fluids of carnivorous plants. The enzyme concentrations used were approximately 10-20 nM pepsin equivalents except for *Drosera* (400 nM), and the concentration of pepstatin was varied from 0 to 120 μM.

In the present study, the hemoglobin-digestion method was not so useful for the assay of the *Drosera capensis* acid proteinase activity; instead, the oxidized insulin B chain was useful as a substrate for the assay as coupled with HPLC. Similar results were also obtained with *D. filiformis*. This may be due mainly to the difference in the substrate specificity of *Drosera* sp. from that of other carnivorous plants and remains to be clarified in a future study. During the course of the present studies, we have also examined the acid proteinase activities of the digestive fluids of some other carnivorous plants. So far, the digestive fluids of *Drosophyllum*

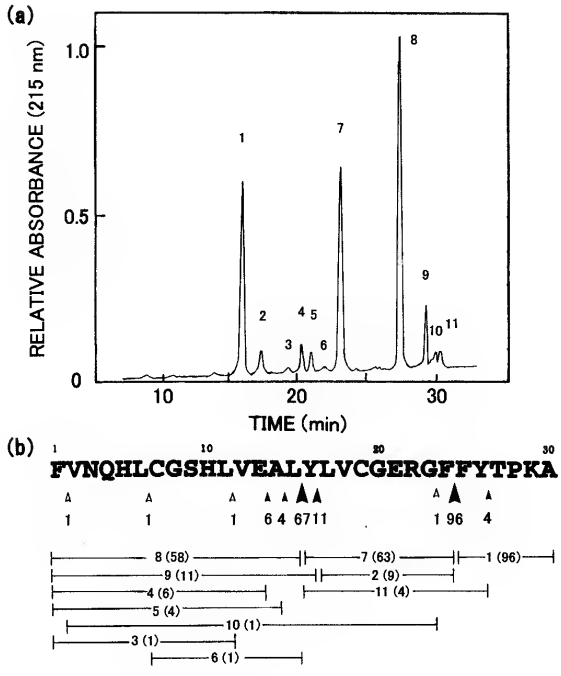

Figure 6: Cleavage specificity of the acid proteinase activity of the digestive fluid of *Nepenthes alata* on oxidized insulin B chain. (a) The chromatogram shows the HPLC pattern of the hydrolysis products. (b) The amino acid sequence of oxidized insulin B chain is given in the one-letter notation. C*, cysteic acid. Large, medium, and small closed arrowheads indicate the major, medium, and minor cleavages, respectively, and small open arrowhead, trace cleavage. The number for each peptide stands for the peak number, and that in parenthesis an approximate value for the relative yield of each peptide. The relative yield was calculated by dividing the peak height by the number of the peptide bonds, assuming the extent at the maximum cleavage site to be 100%.

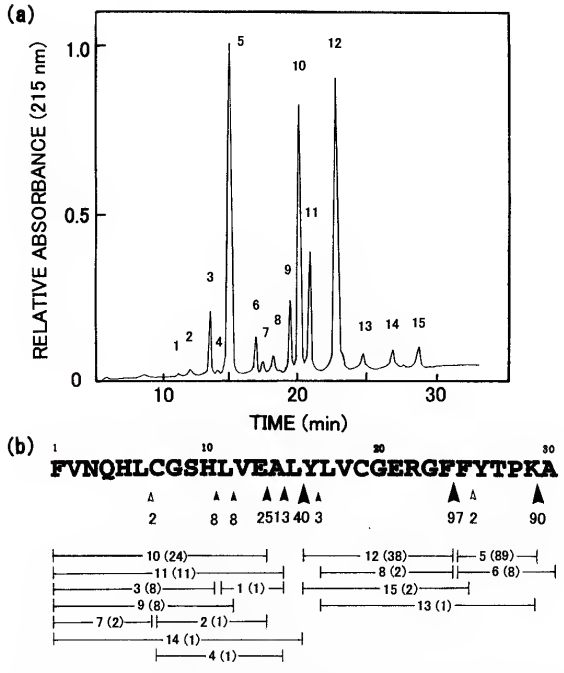

Figure 7: Cleavage specificity of the acid proteinase activity of the digestive fluid of *Cephalotus follicularis* on oxidized insulin B chain. The conditions are the same as those described in the legend to Figure 6.

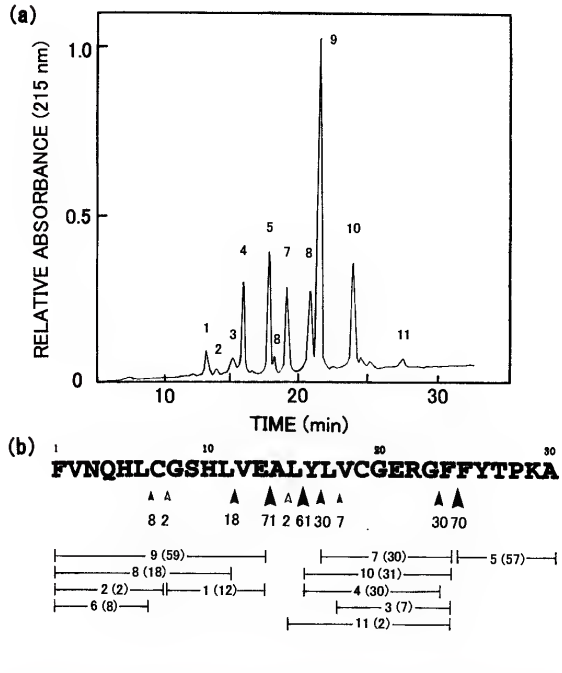

Figure 8: Cleavage specificity of the acid proteinase activity of the digestive fluid of *Dionaea muscipula* on oxidized insulin B chain. The conditions are the same as those described in the legend to Figure 6.

lusitanicum and *Byblis liniflora* showed high activity toward both hemoglobin and oxidized insulin B chain, whereas that of *Sarracenia purpurea* failed to give any activity toward hemoglobin. Further extensive studies along this line, as well as purification and characterization of individual enzymes, including many more carnivorous plants, are necessary for deeper understanding of the biochemistry and physiology of the acid proteinases in the digestive fluids of carnivorous plants.

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Pinguicula elongata of Colombia

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Keywords: observations: Colombia, *Pinguicula elongata.*

I began to study carnivorous plants a few years ago after my botanist friend Randall Peterson gave me a book about carnivorous plants of the world. Ever since I finished my university studies, I have wanted to cultivate and learn more about carnivorous plants, but it is extremely difficult to find seeds or plants in our country.

After two years searching for carnivorous plants in the mountains near Bogotá, the capital of Colombia, with my friend Jairo Ramirez, I found some little red plants glistening in the sun growing on a mountain top.

We took some pictures of these red, healthy plants, some with two or three violet flowers. The plants were growing at an elevation of about 3000 m (9800 ft) in a little depression at the top of the mountain. They were very dispersed, but confined to an area of only about 60 m^2 (645 ft^2) growing with a variety of other plants like grass, frailejón (*Espeletia brassicoidea*, *E. argentea*), lupinius (*Lupinus albus*), romeros (*Diplostephium rosmarinifolium*), piñuelas (*Aechmea* spp.) and others typical of the Páramo, a neotropical high-elevation ecosystem (see Figure 1). The underlying geological formation is a rich sandstone called Formación Guadalupe. Because the area is a declivity, the ground is not waterlogged. The plants may get some water from the typical night fog.

From our photos, I made a drawing of the plant and sent it to Juerg Steiger in Bern, Switzerland. He identified the plant as *Pinguicula elongata*. I also sent some seeds for Juerg to germinate in his greenhouse, but none germinated, despite many attempts.

Pinguicula elongata grows in windy mountain regions that have specific seasonal characteristics. During the short summer season, from December to February, the *Pinguicula* rosettes are dormant, with only short leaves (see Figure 2). During this time, typical daytime temperatures are 28-30°C (82-86°F), and the nighttime temperature drops to 2°C (36°F). There is little or no rain during these summer months, but the nights are foggy. With the first rains in March, the plants revive and produce long leaves that are first pale green to lemon yellow (see Figure 3). In June, the plants change to a deep rusty reddish color (see Figure 4), and the plants produce their first flowers. The leaves and flowers give an incredible contrast with the green monotony of the Páramo. The growing season, March to August, is rainy and the nights are cold and foggy. Temperatures during this time are 5-10°C (41-50°F) during the day, and -2 to +3°C (28-37°F) at night. During September through November the plants slow in growth and prepare for dormancy. Temperatures during this time are 8-15°C (46-59°F) during the day, and 0-5°C (32-41°F) at night, and rains are frequent.

The plants have two flowering seasons, the first is during the winter growing season in June to August, and the second is during November to January, as the plants enter their summer dormancy.

These *Pinguicula* plants are herbaceous perennials with a rosette at the base, elongated leaves with stalked and sessile glands covering the leaf surface. The leaves can grow to 15 cm (6 in), twisting as they ascend, and are covered by the insects caught by the mucilage. The root system is fibrous, but the roots are not long. The flowers grow from the center of the basal rosette. They are white to pale violet with five petals and a backwards pointing spur (see Figure 5). The stigma is white and the stamens are yellow. Each plant can produce three to five flowers. After pollination, the seed capsules turn from brown to black. When the seeds are ripe, the capsules dry and split in the sun, spreading the seeds. When the plants are dormant, they are strongly attached to the ground, and are difficult to find as they hide in the other vegetation.

Several months after our discovery, my son Sebastian found another small population of plants 85 m (280 ft) south, which we called zone no. 2. We have now found two additional areas, both within 200 m (660 ft) of the first two zones. We visit the four zones with my son almost every three months to see the plants, take pictures, and count the plants in the population. We have been spreading seeds to repopulate the *Pinguicula* because these mountains are very fragile. In 2003, a fire destroyed zone no. 1 and we were afraid that the population was lost. But, thanks to the natural seed bank, the population was not affected.

This carnivorous plant population is endemic to this area of the mountains. We have searched other areas having a similar ecosystem, but have found no additional populations. By our spreading seed, we believe we have increased the total population of *Pinguicula* in zones 1-4 by about 40%. We are convinced that this ecosystem needs to be protected and remain hidden from the people that want to reap it to sell these exotic plants.

Because the conditions are exacting and require a controlled environment, few people are capable of growing this kind of carnivorous plant in captivity.

Figure 1: Páramo community; the large plants are *Espeletia* sp., in the Asteraceae. Photo by Heberleyn Hernandez-Melan.

Figure 2: *Pinguicula elongata* in its short-leaved dormancy stage to survive the dry summer. Photo by Heberleyn Hernandez-Melan.

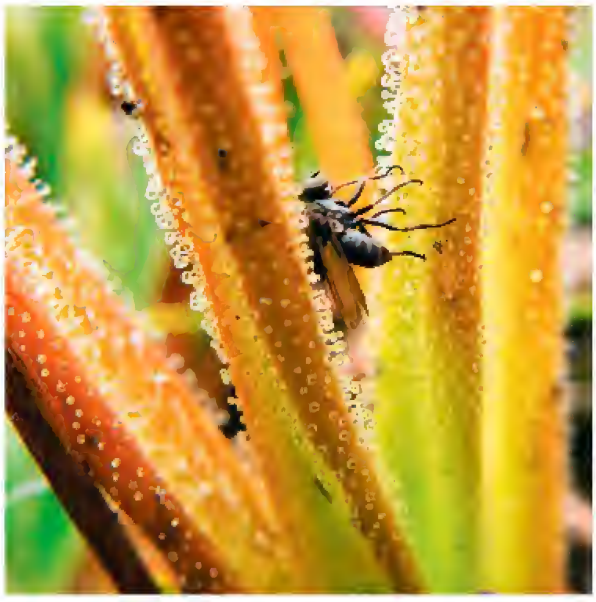

Figure 3:
A fly struggling on the leaf of *Pinguicula elongata*. Photo by Heberleyn Hernandez-Melan.

Figure 4:
Different *Pinguicula elongata* plants, showing a beautiful array of colors. Photo by Heberleyn Hernandez-Melan.

Figure 5:
A white *Pinguicula elongata* flower. The inset shows a more pink flower. The two photographs are not at the same scale. Photo by Heberleyn Hernandez-Melan.

Drosera macrantha subsp. *eremaea*

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Keywords: cultivation: *Drosera macrantha, D. stricticaulis.*

Drosera macrantha is a tuberous *Drosera* native to southeast and southwest regions of Australia and Tasmania (Schlauer 2008). The exact classification of some *D. macrantha* subspecies are under debate, and may be placed under *D. stricticaulis*. For the purposes of this article, I have retained them in *D. macrantha*. *Drosera macrantha* subsp. *eremaea* is a climbing sundew (Cheers 1992) that emerges from the ground in the beginning of autumn. Its tubers have the appearance of a small, round, white egg. In the initial stages of growth, each small sticky leaf takes up to a month to develop into a cup-shape that is green with bright red digestive glands. A month after commencing its growth, the plant grows at a rapid rate of about 1 cm (0.4 in) per day, and soon has a large number of leaves. The internodal length is 3-3.5 cm (1.2-1.4 in). Each leaf node produces a new set of leaves attached to the plant by a thin green stem 5 cm (2 in) in length. After growing for three months, the largest plants in my collection reached its maximum height of approximately 53 cm (21 in) (measured from base of the plant to just below the first flower). The plant produced four flowers; the petals are just over a centimeter in length, are purple in bud, and turn white as they open. The anthers are yellow in colour, which are supported by white filaments and the stigma is green. Pollen granules are yellow, whereas the style and ovary are green. A short pedicel approximately 2 cm (0.8 in) in length supports each flower. Plants do not set seed in my cultivation.

This plant grows very well in pure peat moss (although it is generally recommended that a mixture of peat moss and coarse washed river sand be used for tuberous *Drosera*). My plants receive plenty of direct sunlight throughout the day. Pots should sit in a shallow tray of water. Large pots should be used for growing *Drosera macrantha* as the tubers become pushed down into the growing medium as the plant grows. In addition, tubers multiply during the growing season. Three tubers planted in the same medium sized pot yielded 11 large tubers and three very small tubers at the end of the growing season.

When I last repotted my *D. macrantha* at the end of the season, tubers were placed in new pots containing fresh peat moss and left to dry until next February (the end of summer in Australia). Plants cease growing at the end of spring, usually in October or November. As soon as the plants turn brown, the pot should be removed from the water tray and the growing medium should be allowed to dry out completely during the summer months. During this dormancy period, empty the pot and carefully check the tubers. These can be repotted into a new large pot with the tubers spaced from one another or even planted in separate pots (although it is probably best to place two tubers in the same pot so if one does not grow the other might). Use the same growing medium you used for these same tubers from the previous year's growth.

If you can get them, *Drosera macrantha* may be propagated using seeds, which should be collected and stored until at least one month before autumn when they should be sprinkled onto the surface of the growing medium. Pots should be kept outdoors and kept moist and sitting in water trays. Seeds should sprout in early to mid autumn; usually taking a month or so to sprout. If seeds do not sprout the first year you sowed them, then allow the pot to completely dry out the following spring/summer and then return to the water tray the following autumn where they may finally grow.

A fascinating characteristic of this tuberous *Drosera* is that it produces side shoots from the base of the plant as well as new leaves from some of the leaf nodes. I have observed up to three plantlets growing from the base of one plant. I removed two of these plantlets from the base of an adult plant in the beginning of the winter season, one of these had a large white root that exceeded the actual height of the plantlet's stem and the other had no roots. While both plants grew for a while before dying, neither produced tubers. However, it may be possible to successfully propagate tuberous *Drosera* in this way if cuttings are taken earlier in the growing season.

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Peter D'Amato writes: John Brittnacher's article "Soil Fertilization of *Sarracenia* Seedlings" in Carnivorous Plant Newsletter (CPN) Volume 38, page 36, was quite excellent. At California Carnivores we have also used Osmocote slow-release "pellets" occasionally, particularly on *Nepenthes* that get overhead water and are leached by such overhead watering. We now use Maxsea fertilizer, mostly as a foliar sprinkling, but also occasionally through the soil. We are beginning to use it on all of our carnivorous plants with excellent results. Larry Mellichamp gave an eye-opening slide show at the Atlanta ICPS meeting in 1997, showing the results of soil fertilizing on young *Sarracenia*. And John was quite correct concerning my 1998 article in CPN that overhead sprinkling with diluted fertilizer certainly allows some fertilizer to get into the roots of carnivorous plants (CP), especially short-rooted *Pinguicula*. However I wanted to clarify that the mass death and damage to the Mexican *Pinguicula* was when they were accidentally fertilized using what was then called Mir-Acid, an acidic fertilizer. The damage occurred mostly on the alkaline-loving plants, such as *P. gypsicola* and the like. Most *P. moranensis* were unharmed. I'd be interested to know if John sprays his *Pinguicula* with acidic fertilizers and if he's noticed any damage. I've not had the courage to repeat such an experiment!

8th ICPS Conference in August 2010!

Carnivora, the Dutch Carnivorous Plants Organization, and the Hortus Botanicus Leiden are proud to host the 2010 International Carnivorous Plant Society Conference in Leiden, The Netherlands from the 6th through the 8th of August 2010. The 9th and 10th of August are reserved for optional excursions.

The conference will take place in and within walking distance of one of the world's oldest botanical gardens, located in the heart of historical Leiden. There will be plant sales by international vendors during the conference and an educational exhibition from the 6th till the 16th of August.

Information can be found on the Carnivora website (www.carnivora.nl) by clicking the conference button on the homepage. The website has general information on the botanical gardens, places to stay, what else to see, and other useful travel information.

Registration will also be available on the website when the excursions program and the basic list of lectures are ready, probably around October 2009.

For general questions, contact us at: icps@carnivora.nl

We hope you will be able to join us in The Netherlands.

— Marcel van den Broek

Maurizio Saroldi writes: March 15, 2008: Three friends of mine, Chiara di Biase, Gabriele Basso and Federico Caporlingua, and I left from Italy to Spain with two priorities: finding in Andalucia, in their habitats, *Drosophyllum lusitanicum* and *Pinguicula lusitanica*! We had no GPS data, just some information found on the web about *Drosophyllum* sites and nothing about the small *Pinguicula*; anyway our trip was a complete success!

We were lucky enough to find, near Facinas, a site where hundreds of *P. lusitanica* grew larger, (up to 5 cm (2 in), than those we can find in cultivation! They were half-hidden among the grasses of a wet meadow, in acidic soil composed of peat and sandstone sand. Just a few had their small flowers open. On the last day, on the road from Alcalà de los Gazules to Puerto de Galis, we came upon another small population. There, close to a little spring, in just one square meter (11 sq ft), we found no more than 10 little plants hidden among the grasses. We had more success with *Drosophyllum*. In fact we found 5 sites: Near Los Barrios – a large population with several huge plants, erect as little trees, with wooden stems as large as a finger, and could be 10 years old or more (see Figure 1); Puerto de Galis - smaller plants grew among dwarf-oaks; near Cortes de la Frontera – a large population (see Front Cover). At this site many plants also grew in the shade of the cork-oaks, and some had stems up to 50 cm (19.7 in) long to overcome the surrounding vegetation; near Puerto de las Asomadillas – a few plants; on the road from Alcalá to Puerto de Galis - many very large plants.

We always found *Drosophyllum* in acidic, red sandy-clay soil on sandstone, and in association with *erica, cistus, polygala, lithospermum*, often near cork-oaks. The soil was ever dry. The plants benefit from the morning dew and the long roots reach enough moisture down in the soil during the long dry season. Unfortunately, none were in bloom.

April 25, 2009: Giulio Pandeli and Lucio Semboloni replace Gabriele and Federico and we leave again to Andalucia. In Facinas we once again found *P. lusitanica*, now with many flowers and mature seed capsules. We found *Drosophyllum* in Puerto de Galis, Los Barrios, and Alcalà, and this time it was finally flowering!! We also found *P. vallisnerifolia* and *P. mundii*.

We saw a large population of *P. vallisnerifolia* at the well-known site along Rio Borosa, on the wet and vertical walls of a canyon (see Figure 2). The plants were large, and green, and some were just coming out from dormancy. Just a couple were flowering, but on the other side of the canyon, on a sunny wall, many pale lilac flowers were opened and many plants already had the long leaves typical for this species. We found another population near La Iruela, in a site more difficult to find, where they grew on wet and vertical walls next to a beautiful waterfall. There was a single plant in flower that was of a darker purple colour than in Rio Borosa. Along the walkway that leads to the Rio Mundo source, we found *P. mundii* also growing on dripping, vertical walls, sometimes very close to the waterfall, and seems to withstand surprisingly windy weather. A single plant was in bloom.

We took many pictures and have put many online. Giulio Pandeli also did a great slide show video. The video and images can be found at: http://www.mondocarnivoro.it/andalusia.html

Figure 1: Thick stems of *Drosophylum lusitanicum*. Photo by Maurizio Saroldi

Figure 2: *Pinguicula vallisnerifolia* on a wall in Rio Borosa canyon. Photo by Maurizio Saroldi

New Cultivars

Sarracenia 'Jessica'

Submitted: 5 December 2008

Sarracenia 'Jessica' (see Figure 1) comes from a cross I made between a vigorous green *Sarracenia alata* and one of my *S. leucophylla* clones. Through faster growth and refinement of form and color, this clone soon separated itself from its siblings. *Sarracenia* 'Jessica' produces abundant light-green erect leaves, reaching 56 cm (22 in), that show a yellow suffusion and faint areoles on the upper leaf and the hood. A subdued red-brown venation is also restricted to this area. Older leaves develop lighter coloration and increased translucence on the upper leaf and hood with the veins becoming more prominent. The flower is the feature that distinguishes this cultivar from all other *S.* × *areolata* crosses, and I selected it for cultivar status and named it on 15 May 2007. (see Figure 2)

This is not a strongly colored plant. Rather, *Sarracenia* 'Jessica' exhibits a grace and formal elegance that has made it a constant favorite of mine. On this plant, with its patrician appearance, it always delights and amuses me to see the explosion of gorgeous, incongruous clear-pink flowers that it produces each spring. It is this split personality that has cemented my attachment to this cultivar and which reminded me so much, in a moment of whimsy, of the endlessly complex young woman for which it is named.

To maintain these memorable characteristics, this plant should be reproduced only by vegetative means.

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Figure 1: *Sarracenia* 'Jessica' pitcher. Photo by Jerry Addington.

Figure 2: *Sarracenia* 'Jessica' flower. Photo by Jerry Addington.

The Savage Garden:
Temporary, public displays

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Keywords: cultivation: bog garden

In 2006, my nursery, California Carnivores, was approached by the San Francisco Conservatory of Flowers in Golden Gate Park to lend about 70% of the stock for a six-month carnivorous plant display to be held the following year. The Conservatory plans such large events, with themes such as "Orchids of the World" or "Medicinal Plants", a full year before they occur. I attended a meeting where preliminary plans were laid out. While the Conservatory has always had some carnivorous plants on display, such as *Nepenthes*, in their 100-plus year history, they had never before held a long-term but temporary large event.

The staff at the Conservatory was delightful to work with. The primary focus of the display was to be a rather large bog garden, surrounded by smaller displays. Yet I was quite surprised when they told me they were planning to actually build a large raised container, fill it with wet peat moss and sand, and transplant the plants into it. In other words, build a REAL bog garden! This enormous task perplexed them and horrified me!

No, no, no!" I interrupted. "There's an easier way! You FAKE it!"

The staff looked at me with perplexed expressions.

"I call it the Zip-Lock-Bag-Bog" I told them. I had "invented" this method when California Carnivores did our first large public display in 1990 for the California State Fair in Sacramento.

This is what you basically do: Take established, potted plants, put them in appropriately sized zip-lock bags, and water them over-head so the bag acts as the water tray. You then decorate around the potted, bagged plants with various decorative mosses, such as Oregon Green or Sheet Moss, to hide the pots and make it appear as though the plants were actually planted in soil.

This was a joyous revelation to the staff. It saved them time, there was no sloppy-wet leaky messes, no transplant shock, and best of all it saved a hell of a lot of money. Plus any plant that needed to be removed for whatever reason could be done so and replaced in about two minutes. To keep the plants wet, you simply watered each one separately.

Kristen Natoli, the leader of this project, told me it turned a nightmare into an easy, simplistic pleasure. Many Bay Area Carnivorous Plant Society members, including editors of this Newsletter, also helped make the display a rousing success. Tens of thousands of people saw it. The exhibit was called "Chomp!" While usually the Conservatory never repeats a show for at least ten years, they have asked us to do a "Chomp! 2" exhibit for 2010, only three years after the first.

Temporary, public displays of carnivorous plants are a sure-fire way to publicize this wonderful and growing hobby. If you wish to increase membership of your local carnivorous plant (CP) club or society, its a fun way to do it.

I have done such short-term displays in many places, some quite surprising. My local bank, for instance, has a large wall where they usually exhibit art work, from local professional artists and photographers to children's finger painting. Several times I have done CP exhibits there, but not the Zip-Lock-Bag-Bog. Instead I did a simple terrarium with grow-lights display, what I call the "greenhouse-style" terrarium. This is now of course one of the most popular ways hobbyists grow CP, especially tropicals, at home. But of course even temperate plants like venus flytraps can be grown in tanks seasonally. This is where potted plants are in saucers of water in a tank under grow-lights, as photographed in my book The Savage Garden on page 42 or in Barry Rice's Growing Carnivorous Plants on page 189, where the plants sit on a bed of water-logged sand. You can of course also make such a tank "naturalistic" by using zip-lock bags and mosses, what I call the 'landscaped-potted terrarium".

Whenever I did such a display at my bank, there were always people looking at it in amazement, and all my business cards would be gone. I would have small brief signs offering information about the plants as well.

Here are some places you can set up such temporary, public displays: schools, public libraries, various garden clubs (especially when they have their annual "flower shows"), restaurants (a nice change from aquariums), airports, county fairs, children's museums, even hotel lobbies or popular coffee shops. I even once set up a tank in a dentist's office that mesmerized people for months, and not only did the dentist become a carnivorous plant collector but some of his customers too! (As a gift he gave me a large white pot in the shape of

a molar! *Drosera dichotoma* 'Giant' looks great in it!)

Three times I have done displays in the lobby for productions of "Little Shop of Horrors", now the most popular stage show in America, surpassing the old chestnut "Our Town", if you can believe that!

If you want to increase membership for your local society or club, by all means offer to do a display at local garden centers.....and then tell them how to correctly grow the CP they try to sell! Put up a sign or leave cards or flyers advertising your next meeting. Your membership will certainly increase, and you'll be spreading the intoxicating pleasure all of us already feel by growing these weird and wonderful plants!

Sonoma County Fair prehistoric bog.

THE ICPS SEED BANK

an exclusive member benefit

The International Carnivorous Plant Society offers its members exclusive access to a variety of carnivorous plant seed. For more detailed information about the Seed Bank and a current list of seeds available, check online at:

http://www.carnivorousplants.org/seedbank

If you do not have access to the internet, send a card or letter to the Seed Bank address requesting a copy of the current list (SASE not necessary).

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JOHN BRITTNACHER, Seed Bank Manager, john@carnivorousplants.org

Literature Reviews

By Doug Darnowski

Fleishmann, A. and Rivadavia, F. (2009) *Utricularia rostrata* (Lentibulariaceae), a new species from the Chapada Diamantina, Brazil. Kew Bulletin 64: 155-159.

The authors present a new species of bladderwort from Bahia, Brazil. It occurs in an area of sandstone exposures, and is not only in a protected area but is also fairly abundant. The authors speculate that its only recent identification in spite of its common occurrence is due to its minute size. The description sounds lovely: a tiny species with flowers that vary from purple/mauve to white depending on the amount of light the plant receives. Two of its closest relatives which Carnivorous Plant Newsletter readers might have cultivated are *U. parthenopipes* and *U. blanchetii.*

Libantova, J., Kamarainen, T., Moravcikova, J., Matusikova, I., and Salaj, J. (2009) Detection of chitinolytic enzymes with different substrate specificity in tissues of intact sundew (*Drosera rotundifolia* L.). Molecular Biology Reports 36: 851-856.

While plants can and do make chitinases, it is clear that many carnivores do not make large amount of these enzymes based on the insect exoskeletons, composed of chitin, left behind in many traps once digestion is done. Chitin is also critical in the cell walls of fungi, which can act as plant pathogens, so its production can also be a defense against fungi. This paper shows, using molecular techniques, that *Drosera rotundifolia* produces not only a very wide range of chitinase types but also in many different tissues throughout the plants, Since many of these tissues are internal and/or not part of leaves, it is clear that at least many of these chitinases are not involved in carnivory in *D. rotundifolia*, though exactly what they are doing for the plants in these locations is not yet known.

Bove, C.P. (2008) A new species of *Utricularia* (Lentibulariaceae) from central Brazil. *Revista Brazil.* Bot. 31: 555-558.

In this paper the author describes *Utricularia cochleata*, a new species of bladderwort from Brazil, notable for growing on rocks covered by liverworts and moss, watered by mist from a waterfall. It is most closely related to *U. aureomaculata* and *U. steyermarkii*. The flowers of deep yellow and the challenge of cultivating a lithophyte (see Barry Rice's book on which groups of bladderwort species are most commonly cultivated, by natural habitat type) may tempt some ICPS members to give it a try if and when it becomes available in the trade.